The effects of two different intensities of aerobic training protocols on pain and serum neuro-biomarkers in women migraineurs: a randomized controlled trail

Eslami, Rasoul ; Parnow, Abdolhossein ; Pairo, Zahra ; Nikolaidis, Pantelis ; Knechtle, Beat

Abstract: Objectives: We have a weak understanding of how aerobic training may influence migraine, and the optimal parameters for exercise regimens as migraine therapy are not clear. The objectives of this study were to assess, first, effects of two different intensities of aerobic exercise on migraine headache indices; second, serum neuro-biomarker in women migraineurs. Methods: A total of 45 non-athlete female migraine patients were selected by a neurologist and randomly divided into three groups: control (CON), moderate-intensity aerobic training (MOD T), and high-intensity aerobic training (HIGH T). Before and after the training protocol, body composition factors, migraine pain indices, VO2max, and serum Adenylate-Cyclase Activating Polypeptide (PACAP) and Substance P (SP) were measured. Exercise training protocol includes two different intensities of aerobic exercise: Moderate (13-15 Borg Scale, 60-80% HRmax) and High (15-17 Borg Scale, 65-95% HRmax). Results: Moderate-intensity aerobic training (MOD T) reduced headache intensity, frequency, and duration in women with migraine ($p < 0.001$, for all). Also, high-intensity aerobic training (HIGH T) reduced headache intensity, frequency, and duration ($p < 0.001$, for all). However, for headache intensity and duration, MOD T was effective rather than HIGH T ($p < 0.001$; $p \leq 0.05$, respectively). In addition, neither MOD T nor HIGH T could not alter PACAP and SP contents ($p = 0.712$; $p = 0.249$, respectively). Conclusions: Our results demonstrated that either MOD T or HIGH T could modify migraine pain indices but neither MOD T nor HIGH T could not alter the PACAP and SP contents in women with migraine.

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The Effects of Two Different Intensities of Aerobic Training Protocols on Pain and Serum Neuro-biomarkers in Women Migraineurs: A Randomized Controlled Trail

Different intensities of aerobic training and migraine pain

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Abstract

Objectives: We have a weak understanding of how aerobic training may influence migraine, and the optimal parameters for exercise regimens as migraine therapy are not clear. The objectives of this study were to assess, first, effects of two different intensities of aerobic exercise on migraine headache indices; second, serum neuro-biomarker in women migraineurs.

Methods: A total of 45 non-athlete female migraine patients were selected by a neurologist and randomly divided into three groups: control (CON), moderate-intensity aerobic training (MOD T), and high-intensity aerobic training (HIGH T). Before and after the training protocol, body composition factors, migraine pain indices, VO_2max, and serum Adenylate-Cyclase Activating Polypeptide (PACAP) and Substance P (SP) were measured. Exercise training protocol includes two different intensities of aerobic exercise: Moderate (13-15 Borg Scale, 60% to 80% HRmax) and High (15-17 Borg Scale, 65% to 95% HRmax).

Results: Moderate-intensity aerobic training (MOD T) reduced headache intensity, frequency, and duration in women with migraine ($p<0.001$, for all). Also, high-intensity aerobic training (HIGH T) reduced headache intensity, frequency, and duration ($p<0.001$, for all). However, for headache intensity and duration, MOD T was effective rather than HIGH T ($p<0.001$; $p\leq0.05$, respectively). In addition, neither MOD T nor HIGH T could not alter PACAP and SP contents ($p=0.712$; $p=0.249$, respectively).

Conclusions: Our results demonstrated that either MOD T or HIGH T could modify migraine pain indices but neither MOD T nor HIGH T could not alter the PACAP and SP contents in women with migraine.

Key words: Migraine, Aerobic Training, Adenylate-Cyclase Activating Polypeptide (PACAP), Substance P (SP)

Abbreviations:

BMI: Body Mass Index

CGRP: Calcitonin Gene-related Peptide

CON: Control

CRP: C-Reactive Protein

eCB: endocannabinoid

HRmax: Heart Rate Maximum

HIGH T: High-intensity aerobic training

IL-6: Interleukine-6

NPY: Neuropeptide Y

NO: Nitric Oxide

MOD T: Moderate-intensity aerobic training

PACAP: Pituitary Adenylate-Cyclase Activating Polypeptide

RCT: Randomized Controlled Trial

SNL: Spinal Nerve Ligation

SP: Substance P

VAS: Visual Analog Scale

Vo2max: Maximum Volume of Oxygen Consumption

WHR: Waist-to-Hip Ratio

Introduction

Migraine is a debilitating neurovascular disorder characterized by severe headache attacks often associated with autonomic and neurological symptoms (Ferrari, 1998; Goadsby, Lipton, & Ferrari, 2002). Depression, chronic fatigue, disability on work, family problems, and drug dependency are some psychosocial problems related to these chronic headaches (Gantenbein, Afra, Jenni, & Sándor, 2012). It is well established that migraine headache has a higher prevalence in females than males. Many studies with a variety of samples and methodologies revealed that females have a higher prevalence ratio in migraines than males (Benbir et al, 2012; Henry et al,1999; Kim et al, 2013; Buse et al, 2013). Also, females may report more symptoms and headache-related disability (Benbir et al, 2012; Henry et al,1999; Steiner et al, 2003).

The lack of suitable therapeutic modalities in managing migraine reflects the limited understanding of the pathological mechanisms behind it. According to the neurovascular theory of migraine, several neuropeptides may play a role in trigemino-neurovascular system activation, which has been considered as one of the important migraine pathomechanism (Tajti, Szok, Majláth, Tuka, & Csáti, 2015). Several studies in this regard have reported the role of neuropeptides, including calcitonin gene-related peptide (CGRP), Nitric Oxide (NO), Neuropeptide Y (NPY), Pituitary Adenylate-Cyclase Activating Polypeptide (PACAP) and Substance P (SP), in migraine pathophysiology (Tajti et al., 2015).

Adenylate-Cyclase Activating Polypeptide (PACAP) is a multifunctional vasodilator peptide that has recently been shown to play an important role in migraine pathogenesis, such as CGRP. A new clinical study has provided evidence of a clear association between migraine phases (ictal and interictal) and plasma PACAP alterations in migraine (Tuka et al., 2013). This study showed that the plasma levels of PACAP were increased during migraine attacks compared to their interictal levels, while interictal PACAP levels were lower in comparison to healthy subjects (Tuka et al., 2013). Also, peripheral injection of PACAP in migraineurs, induced a delayed migraine-like headache, while PACAP injection in healthy subjects led to initial headache (Schytz et al., 2009). Therefore, PACAP appears to have the ability to induce a delayed migraine-like headache, such as CGRP.

SP is a member of the tachykinin-neuropeptide family (Chang, Leeman, & Niall, 1971). During activation of the trigemino-neurovascular system, SP induces vasodilatation and plasma protein extra vacation in the cerebral dura mater (Buzzi & Moskowitz, 1990; Moskowitz, 1993). In the clinical situation, the salivary SP immunoreactivity was elevated during spontaneous migraine attacks without aura when compared with the control subjects (Nicolodi & Del Bianco, 1990). Also, in chronic migraine patients, the saliva and plasma levels of SP were higher than those in healthy subjects, and associated with pain intensity (Jang, Park, Kho, Chung, & Chung, 2011). Therefore, recent evidence focuses on the possible role of PACAP and SP in migraine pathophysiology.

Aerobic (endurance) exercise is considered as a therapeutic method of reducing symptoms of several chronic diseases and medical conditions (Scheef et al., 2012). As direct evidence, the therapeutic effects of endurance training on migraine have been reported in numerous studies. A large body of literature addressing exercise may be particularly helpful in reducing the frequency, duration, and intensity of migraine headaches (Darabaneanu et al., 2011; Varkey E, 2009; Varkey, Cider, Carlsson, & Linde, 2011). However, the number of studies describing the mechanisms of exercise beneficial effects on migraine are few and limited to CGRP, NO, Beta-endorphin, and, in some cases, other neuropeptides (Maeda et al., 2001; Onuoha, Nicholls, Patterson, & Beringer, 1998). For example, it has been found that beta-endorphin levels were lower in patients with migraine in comparison to healthy controls (Misra et al., 2013) where exercise resulted in increased beta-endorphin levels and consequently resulted in lower headache days (Köseoglu et al., 2003; Amin et al., 2018). However, no studies have looked at variations in PACAP and SP in migraine patients who have exercised. Therefore, the effects of exercise training biomarkers such as PACAP and SP, involved in migraine pathophysiology are not yet clear and requires further evaluation. On the other hand, when designing an appropriate aerobic program, exercise duration and intensity are usually manipulated. Because there are different aerobic training protocols to execute, identification of effective training intensity and volume (workload) is a very important issue in exercise training prescription.

Given the limitations of previous studies about headache improving mediated by exercise training in the migraine society and the unknown mechanism involved in this process are strong reasons why future investigations should be performed. Consequently, the purpose of the current study was to estimate the effects of two different aerobic protocols on headache indices and to investigate the behavior of serum levels of biological markers (PACAP and SP) playing a key role in pain mechanisms, after aerobic training protocols in women migraineurs. The research hypothesis, therefore, was that the intensity of training is a main variable to determine a positive effect of MOD and HIGH aerobic training on headache indices and to be effective to decrease migraine pain indexes in migraine patients.

Material and Methods

Research design

This was a randomized controlled clinical trial (RCT) with a semi-experimental design (three groups in the form of pre-test and post-test) (RCT code: IRCT2015100910824N2) and was conducted among the migraine patient's community in Kermanshah city, Iran. The experimental procedures and study protocols were approved by the Ethics Committee of Kermanshah University of Medical Sciences (ethical code: kums.rec.1394.15).

In order to determine the number of subjects, the randomized sampling method was used. Volunteers were recruited from neurology clinics, and neurology center of Kermanshah hospital (Kermanshah, Iran). The inclusion criteria were as follows: (1) Not having a history of regular exercise in the last six months, (2) having no cardiovascular disease, hypertension, diabetes, arthritis, cancer, asthma, AIDS, meningitis and Multiple Sclerosis, (3) confirming headache by the neurologist, and (4) obtaining the entry criteria for the VO_2max test (above 20 ml /kg /min). Exclusion criteria were including: (1) situations that researchers have understood during research period such as pregnancy, breastfeeding, abuse of drugs or alcohol; (2) involving in any extra exercise training programs; (3) not interested to continue or change in personal life schedule.

According to the study criteria for subject selection, 45 non-athlete female migraine patients were selected by the neurologist. Prior to participation, written consent was obtained from all patients. The subjects who met the inclusion criteria were randomly assigned into three groups: control (CON) (*n*=15), moderate-intensity aerobic training (MOD T) (*n*=15), and high-intensity aerobic training (HIGH T) (*n*=15) (**Figure 1**). The initial demographic characteristics of the subjects for the different groups are shown in **Table 1**. Patients in Control group (CON) were instructed to keep a diary regarding exercise during the experiments period. Experimental groups included MOD T and HIGH T which MOD T group executed moderate aerobic training and HIGH T group executed high-intensity aerobic training. All three groups received their drug treatment under neurologist supervision.

The main protocol of this study lasted for four months: 1 month of migraine evaluation, 2 months of training, and 1 month of post evaluation. One month before and after the protocol, the migraine headache indexes (*i.e.* frequency, duration, and intensity of the attacks) were evaluated by daily headache record questionnaire (Darabaneanu et al., 2011; Narin, Pinar, Erbas, Oztürk, & Idiman, 2003). In addition, 24 h before and 48 h after the training protocol, body composition factors (height, age, weight, fat percentage) (In body; Model: Zuse 9.9, South Korea), body mass index (BMI), waist-to-hip ratio (WHR) and VO_2max (Astrand Bicycle, Monark E839, Sweden) were measured.

Pain evaluation questionnaire

The pain was evaluated using a visual analog scale (VAS). In this scale, the amount of pain that a patient feels ranges across a continuum from none to an extreme amount of pain (the number 0 without pain and the number 10 indicates exacerbation of the pain). The mean duration of headache (min per month), as well as the frequency of headache (days per month) was evaluated using this questionnaire (Narin, Pinar, Erbas, Oztürk, & Idiman, 2003).

PACAP and SP plasma levels measurements

One day before starting training protocols and 48 h after the last training sessions, blood samples were collected from an cubital vein at the same time in the morning and after a 12 h fasting state. For all groups, blood samples were collected outside a migraine attack and having taken no symptomatic medication the day before. Blood samples were immediately centrifuged at a temperature of 4 °C and 3000 rpm for 10 min. Thereafter, plasma was separated and kept at -80 °C until analysis. Plasma levels of PACAP and SP were measured by commercially available enzyme-linked immunosorbent assay kits (Kazabu Co, Japan, with catalog number CSB-E09348h for PACAP; R & D Co, Canada, with catalog number KGE007 for SP) according to the procedures provided by the manufacturer.

Exercise Training protocols

As mentioned above, in this study, we have two experimental groups. These two groups not only received their drug treatment but were also treated with aerobic training including MOD T and HIGH T. As shown in Table 2, the MOD T protocol (13-15 Borg Pressure Understanding Scale, 60-80 % heart rate max, equivalent to 45% -70 % VO_2max) and HIGH T protocol (15-17 Borg Pressure Understanding Scale, 65-95 % heart rate max, equivalent to 55% - 90% VO_2max) were designed from a simple to heavy state. A simple or heavy state was declared by subjects according to training pressure. The training programs included eight weeks, three sessions per week, 30-60 min per session including warm up and cool down. For both training groups, every training session was divided into three parts: A. warming up (10 min), B. main training (according to weekly duration), and C. cooling down (10 min) (Table 2).

Drug administration

It is important to mention that during study, all patients used and monitored migraine medications according to expert neurologist. Based on initial assessment by expert neurologist, needed dose of drug (including Diclofenac, Nortriptyline and Duloxetine, Sumatriptan, Depakin, Propranolol, Dexamethazone, Venlafaxine, Gabapentin, Ergotamine, Zolpidem, Codein, Ibuprofen) was administered to all patients and dose of drug used were recorded during study.

Statistical analysis

Data were expressed as mean ±SD. Distribution of data was assessed for normality using the Shapiro—Wilk test. The data were normally distributed, therefore, were analyzed using parametric methods. A 2 (Time) x 3 (Group) mixed-model of Repeated measures ANOVA test, followed by paired sample t-test (for pre-post comparison in every group) and Scheffe post hoc test (for between-groups comparison) were used. The differences were accepted as significant if $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$.

Results

Physiological indexes

A 2 (Time) x 3 (Group) mixed-model ANOVA for weight data analysis revealed that the main effect for time was not significant ($F(1,30) = 4.934$, $p = 0.081$, Eta-squared = 0.098). This means weight was not different between pre and post time, for three groups. Also, no significant main effect for group was obtained ($F(1,30) = 2.851$, $p = 0.074$, Eta-squared = 0.160). Thus, there was no overall difference in the weight between the three groups. However, Time x group interaction effects was significant ($F(2,30) = 10.157$, $p < 0.0001$, Eta-squared = 0.404). A comparison of means indicated that weight was decreased after aerobic training only in the MOD T group, in comparison to other groups (Table 3).

In addition, data analysis of BMI showed that the main effect for group was significant ($F(2,30) = 4.802$, $p = 0.016$, Eta-squared = 0.243). Thus, there was a significant difference between the three groups. The pairwise comparison test showed that BMI in the MOD T group was significantly higher than the HIGH T group ($p<0.05$). Also, no significant main effect for Time was obtained ($F(1,30) = 3.317$, $p = 0.079$, Eta-squared = 0.100). This means BMI was not different between pre and post time for the three groups. However, Time x group interaction effects was significant ($F(2,30) = 10.04$, $p < 0.0001$, Eta-squared = 0.401). A comparison of means indicated that BMI decreased after aerobic training only in the MOD T group, in comparison to other groups (Table 3). The results of repeated measures ANOVA for body fat percent revealed a significant main effect for Time ($F(1,30) = 13.59$, $p \leq 0.001$, Eta-Squared = 0.312). Pairwise comparison test showed that in MOD T and HIGH T groups post-test body fat percent was significantly lower than pre-test ($p < 0.001$; $p \leq 0.05$, respectively). This indicating that both MOD T and HIGH T were effective in body fat percent reduction in women with migraine disorder (Table 3). In addition, the main effect for group was not significant ($F_{(2,30)} = 3.290$, $p = 0.051$, Eta-squared = 0.180). Thus, there was no overall difference between the three groups for body fat percent. However, a significant Time x Group interaction effects was also obtained, $F_{(2,30)} = 6.622$, $p < 0.01$, Eta-squared = 0.306). A comparison of means indicated that body fat percent was decreased after aerobic training only in the MOD T group, in comparison to other groups (Table 3).

For VO_2max, firstly, it was compared initial VO_2max of patient between three groups via statistical ANOVA test where there was not any significant difference between the three groups ($p = 0.088$). Results showed that there was a significant main effect for Time ($F_{(1,30)} = 35.114$, $p < 0.0001$, Eta-Squared= 0.539). The pairwise comparison test showed a significant difference between pre-test and post-test in MOD T and HIGH T groups ($p < 0.001$; $p \leq 0.001$, respectively). This indicating that both MOD T and HIGH T were effective in VO_2max improvement in women with migraine disorder; although the main effect for group was not significant ($F_{(2,30)} = 2.658$, $p = 0.087$, Eta-squared = 0.151). Thus, there was no overall difference between the three groups for VO_2max. However, a significant Time x Group interaction effects was also obtained, $F_{(2,30)} = 17.434$, $p < 0.0001$, Eta-squared = 0.538). A comparison of means indicated that both MOD T and HIGH T groups had a significant increase in VO_2max after aerobic training rather, in comparison to the CON group, however, there was not a significant difference between MOD T and HIGH T groups (Table 3).

In addition, for WHR, the main effect for group was not significant ($F_{(2,30)} = 0.628$, $p = 0.540$, Eta-squared = 0.040). Thus, there was no overall difference between the three groups. Also, no significant main effect for Time was obtained ($F_{(1,30)} = 0.657$, $p = 0.424$, Eta-squared = 0.021). This means WHR was not different between pre and post time, for three groups. Moreover, Time x group interaction effects was not significant ($F_{(2,30)} = 1.626$, $p=0.214$, Eta-squared = 0.098). Therefore, WHR after the two aerobic training was not different than before aerobic training (Table 3).

Migraine pain indexes

A 2 (Time) x 3 (Group) mixed-model ANOVA revealed that there was a strong main effect for Time ($F_{(1,29)} = 62.83$, $p < 0.001$, Eta-Squared = 0.684). pairwise comparison test showed that in MOD T and HIGH T groups, intensity score was significantly lower in post-test rather than pre-test ($p < 0.001$; $p \leq 0.001$, respectively). This indicated that both MOD T and HIGH T were effective in pain intensity reduction in women with migraine disorder. In addition, the main effect for group was not significant ($F_{(2,29)} = 0.389$, $p = 0.681$, Eta-squared = 0.026). Thus, there was no overall difference between the three groups ($p = 0.681$). However, a significant Time x Group interaction effects was also obtained, $F_{(2,29)} = 12.241$, $p < 0.001$, Eta-squared = 0.458). Comparison of means indicated that, in comparison to the CON group, both MOD T and HIGH T groups had lower intensity the score after aerobic training rather than before aerobic training, however intensity decrease in the MOD T group was larger than HIGH T group. This means moderate-intensity aerobic training was more effective to decrease pain intensity (Table 3).

In addition, for frequency, there was a strong main effect for Time ($F(1,29) = 85.01$, $p < 0.001$, Eta-Squared = 0.746). The pairwise comparison test showed that, for both MOD T and HIGH T groups, the frequency score in the post-test was significantly lower than the pre-test ($p < 0.001$; $p \leq 0.001$, respectively). This indicated that both MOD T and HIGH T were effective to pain frequency reduction in women with migraine. In addition, the main effect for group was significant ($F(2,29) = 4.411$, $p < 0.05$, Eta-squared = 0.233). The pairwise comparison test showed that the frequency score in MOD T and HIGH T groups was significantly lower than the CON group ($p < 0.01$; $p < 0.05$, respectively). Also, there was not any difference between MOD T and HIGH T groups ($p = 0.462$). Moreover, a significant Time x Group interaction effects was also obtained, $F(2,29) = 12.241$, $p \leq 0.001$, Eta-squared = 0.384). Comparison of means indicated that, in comparison to the CON group, both MOD T and HIGH T groups had a lower frequency the score after aerobic training rather than before aerobic training, however, there was not a significant difference between MOD T and HIGH T groups (Table 3).

In addition, for Duration, a significant strong main effect for Time was obtained ($F(1,29) = 54.47$, $p < 0.001$, Eta-Squared = 0.653). The pairwise comparison test showed that, for CON, MOD T, and HIGH T groups, the Duration score in the post-test was significantly lower than the pre-test ($p<0.001$, for all). This means migraine duration was decreased in three groups. In addition, the main effects for Group was significant ($F(2,29) = 4.872$, $p < 0.05$, Eta-squared = 0.252). The pairwise comparison test showed that Duration in the HIGH T group was significantly lower than the CON group ($p < 0.05$). Also, there was not any difference between MOD T and HIGH T groups ($p = 0.99$). Moreover, a significant Time x Group interaction effects was also obtained, $F(2,29) = 3.743$, $p \leq 0.05$, Eta-squared = 0.205). A comparison of means indicated that both MOD T and HIGH T groups had a significant decrease in duration score after aerobic training rather than before aerobic training, in comparison to the CON group. However, the decrease in the MOD T group was larger than the HIGH T group. This means moderate-intensity aerobic training was more effective to decrease pain duration (Table 3).

PACAP and SP

For PACAP, a 2 (Time) x 3 (Group) mixed-model ANOVA revealed that the main effect for group was not significant (F (2,30) = 1.95, p = 0.160, Eta-squared = 0.115). Thus, there was no overall difference between the three groups. Also, no significant main effect for time was obtained (F (1,30) = 1.472, p = 0.234, Eta-squared = 0.047). This means PACAP levels were not different between pre and post time, for three groups. Moreover, time x group interaction effects were not significant (F (2,30) = 0.344, p = 0.712, Eta-squared = 0.022). Therefore, PACAP after the two aerobic training was not different than before aerobic training (Table 3) (Figure 2).

In addition, data analysis of SP showed that the main effect for group was not significant (F (2,30) = 0.490, p = 0.618, Eta-squared = 0.032). Thus, there was no overall difference in the SP between the three groups. Also, no significant main effect for Time was obtained (F (1,30) = 1.43, p = 0.241, Eta-squared = 0.046). This means SP levels were not different between pre and post time, for three groups. Moreover, Time x group interaction effects was not significant (F (2,30) = 1.456, p = 0.249, Eta-squared = 0.089). Therefore, SP after the two aerobic training was not different than before aerobic training, in comparison to the control group (Table 3) (Figure 2).

The research hypothesis, therefore, was that aerobic training with different intensities could determine the role of volume and intensity of exercise training with a positive effect on headache indices as well as serum levels of PACAP and SP in migraine patients.

Discussion

The purpose of the current study was the effects of two different intensities (MOD T and HIGH T) of aerobic training protocols on headache indexes and to investigate them on a serum levels of biological markers (PACAP and SP) in women migraineurs. It was found that, after 8 weeks of MOD T, the intensity (from 7.38 to 3.73 vas), the duration (from 8.68 to 3.6 hours per month), and the frequency (from 7.54 to 2.27 times per month) of migraine headaches decreased and after 8 weeks of HIGH T, the intensity (from 5.79 to 4.22 vas), the duration (from 7.29 to 4.04 hours per month), and the frequency (from 8.08 to 3.25 times per month) of migraine headaches reduced.

So far, several studies have been conducted regarding exercise training and migraine pain which their results support the positive effects of aerobic training on migraine pain indicators (intensity, duration, and frequency). For example, Kroll et al. (2018) revealed that 45 min of aerobic exercise, 3 times per week, for 3 months lead to a reduction of pain intensity, pain duration, and frequency of migraine attacks. Narin et al. (2003) reported that 8 weeks of aerobic training (3 sessions per week and one hour per session) reduced the intensity of migraine headaches (from 8.8 to 0.4 vas), frequency of headaches (from 4.7 to 3.6 times per month), and duration of headaches (from 37.4 to 11.4 h) in migraine patients (Narin, Pinar, Erbas, Oztürk, & Idiman, 2003). These researchers attributed the improvement in migraine pain indices to the increase of plasma nitric oxide (Narin et al., 2003). Also, Darabaneanu et al. (2013) conducted a study that included an aerobic exercise protocol as running for 10 weeks. They reported a significant decline in headache intensity (from 0.7 to 0.4 vas), headache frequency (from 3.8 to 2.3 times per month), and duration of headache (from 0.9 to 0.4 h per month), and these can be related to increase in physical fitness of patients (Darabaneanu et al., 2011).

However, a number of studies could not demonstrate the effects of aerobic training on migraine headache indices. In terms of the intensity of headache, Loket et al. (1992) and Grimm et al (1981) reported that 6 weeks of aerobic training (3 sessions per week, 50 to 85% VO_2max) did not have any positive effect on migraine pain intensity (Grimm, Douglas, & Hanson, 1981; Lockett & Campbell, 1992). In these two studies, the period of training was 6 weeks that may be too short to produce a sufficient physiological adaptation. In terms of headache duration, Dittrich et al. (2008) did not show the effects of aerobic training on headache duration. Their exercise training program contains 6-week aerobic training with 2 sessions per week, which included 45 min of gymnastics with music and 15 min of relaxation (Dittrich et al., 2008). They used a training program with two sessions per week that, according to exercise training principles, cannot induce a complete sensitivity to training. In terms of the frequency of headache attacks, it was reported that 12 weeks of aerobic cycling for three days per week and 20 min, each session did not show any significant effect on the frequency of headache attacks (Norlander, Cider, Carlsson, & Linde, 2007). In this study, the time of training per session was very short (20 min) that may be insufficient to induce training effects. Therefore, according to the training protocols used in previous studies, it seems that low frequency exercise training per week, short duration exercise per session, short term period (less than 6 weeks), or low total work volume per week (time*frequency) are likely important reasons for the ineffectiveness of these training programs. However, in this study, we an used aerobic training program including 3 sessions per week for 8 weeks with 30-40 min per session which thought is sufficient to induce physiological and functional improvement in patient with migraine.

In this study, we tried to investigate about this question "which aerobic training intensity is more appropriate for improving migraine pain indexes?" Therefore, we used two different intensity of aerobic training (MOD T (45-70% VO_2max) and HIGH T (55-90% VO_2max) with different training volume. The results of this study showed that the intensity of aerobic training was not the main factor in migraine pain control. This is reason why both moderate and high intensity were able to improve the parameters of migraine pain, although the moderate-intensity was somewhat more effective. Therefore, according to the results of this study, it seems that one of the main factors for the success of aerobic programs to improve the Migraine pain indices is its frequency and weekly repetitions. In other words, aerobic training with more than 3 days per week and with moderate to high-intensity levels can be useful for improving migraine pain indicators (intensity, duration, and frequency of headache attacks). In this regard, health guidelines also have reported 150 min of average intensity aerobic training and 75 min of high-intensity aerobic exercises per week with a frequency of 3 to 5 sessions as a useful aerobic training program for health and physical fitness (Kaminsky, 2014). Our results also revealed that MOD T was more effective to decrease migraine pain indexes, although we expect high intensity does it. As the intensity was higher in HIGH T and the volume was higher in MOD T, the workload was calculated via time * RPE and results showed both protocols made exertion the same workload. These results indicate that both MOD T and HIGH T could be an important alternative method in monitoring headache attacks and there is no problem when patients use HIGH T and they could save their time since the time of HIGH T is shorter than MOD T. One of the differences between MOD and HIGH T probably depends on muscle fibers recruitments with showing Slow Oxidative (So) muscle fibers more involved in MOD T and Fast Oxidative Glycolytic (FOG) muscles fibers involved in HIGH T.

In addition, our results showed that both moderate and high-intensity aerobic training improve VO_2max and decrease body fat percentage in women with migraine. Also, MOD T could modify weight and BMI in women migraineurs. However, WHR was not affected by neither MOD T nor HIGH T. Thus, aerobic training, especially MOD T, could improve both physical fitness and health factors in women with migraines. Exercise-induced aerobic fitness can affect the physiology of migraine in multiple ways. For example, aerobic training manage weight that result in self-esteem and self-confidence in migraine patients. The body fat percentage reduction also leads to decrease inflammatory factors (*e.g.,* IL-6 and CRP) including in migraine pathophysiology (Irby, et al., 2016). Beyond neurophysiologic pathways, improvements in aerobic fitness and migraine likely are mediated by overlapping alter in behavioral and socio-cognitive factors (*e.g.,* self-efficacy beliefs, outcome expectancies) (Irby et al., 2016; Hindiyeh, Krusz & Cowan, 2013). However, we run correlation test between VO_2max and migraine indexes in post training, but our results showed no significant correlations.

There are different mechanisms contributing to the reduction of migraine pain indices after exercise training. For example, Endogenous Opioids System, endocannabinoid (eCB), CGRP, and neurotrophic brain-derived factors may play a significant role in the regulation of pain signaling, which can increase their functions following aerobic exercise training and result in pain relief (Hindiyeh, Krusz, & Cowan, 2013). Beta-endorphin is an endogenous opioid with analgesia effects (Guillemin et al., 1977). Its levels have been found to be lower in patients with migraine in comparison to healthy control. Also, it is lower during migraine attacks than in pain-free periods (Anselmi et al., 1980). It seems that beta-endorphin inhibits the release of SP which results in a lower function of pain pathways (Brunton, 2006). However, exercise can increase beta-endorphin levels. In this regard, Köseoglu et al. (2003) studied 6 weeks of aerobic exercise (40–50 min 3 times per week at 60–80% of maximal heart rate) on female migraine without aura patients during headache-free periods. They reported that the aerobic exercise increases the beta-endorphin levels which possibly lead to fewer headache days. PACAP also has been reported to be antinociceptive in the periphery (Sandor et al., 2009, 2010). One study reported that inhibition of PACAP expression in the dorsal horn may relieve pain in patients or animals with neuropathic pain (Mabochi et al., 2004). And, Yamaoka et al. (2017) reported that exercise therapy as a force treadmill running possibly involves pain relief in spinal nerve ligation (SNL) rats by suppressing PACAP expression in the spinal cord. They concluded that altered gene expression of PACAP possibly involved in the mechanism of exercise-induced analgesia for neuropathic pain in rats.

In the present study, also, the effects of different intensity of aerobic training on the biological indices involved in migraine headaches were investigated. Therefore, we selected two neuropeptides involved in the pathophysiology of migraine, PACAP, and SP, which their role has recently been reported in migraine headache. According to our review of databases, not any study has been carried out to investigate the effect of exercise training on serum levels of PACAP in migraine patients. In other words, we first reported the effect of exercise training as aerobic form on serum levels of PACAP in migraine patients. PACAP has been shown to play a role in the pathogenesis of migraine, such as the CGRP (Schytz et al., 2009; Tuka et al., 2013). However, despite our expectation, the results of the present study showed that 8 weeks of both aerobic training protocols, MOD T and HIGH T, did not affect the serum levels of PACAP in migraine patients. Previous studies have shown that PACAP alterations in migraine patients were two-dimensional when compared with healthy subjects. The quantitative changes in this peptide in the plasma are related to the disease duration. PACAP-38 concentration has been shown significantly lower in the interictal plasma of the migraineurs as compared with the healthy controls and plasma and significantly higher in the ictal phase relative to the attack-free (Tuka et al., 2013). In this study, significantly lower PACAP-38-LI was determined in the interictal plasma of the migraineurs (n=80; 24.60±3.59 fmol/ml) than in that of the healthy volunteers (n=40; 26.54±4.43 fmol/ml). However, the plasma samples from the patients during their migraine attacks (n=28) exhibited a significantly higher PACAP-38 concentration (27.39±4.67fmol/ml) as compared with the interictal samples (n=59; 24.91±3.73 fmol/ml) (Tuka et al., 2013).

Therefore, the pattern of PACAP alteration in migraine patients is a sinusoidal and does not have a consistent level in these patients. In the present study, we used 8 weeks aerobic training protocols, which included a number of these attack periods and between attacks periods. Although, patient's migraine state in moment of blood collection may has a critical role in plasma PACAP level, however, in our study, it was not possible to collected blood samples in same migraine phase (ictal or interictal) since time of last training session until blood collection moment must to be same for all subjects. It, therefore, is likely that, in moment of blood collection, some of subjects were in ictal phase and some other were in interictal phase. Therefore, the sinus pattern of the PACAP alteration pattern can be one of the possible reasons for the unchanging it after aerobic training protocols. It was suggested that the effect of aerobic training on serum PACAP would be investigated in separated periods of ictal and interictal.

Another possibility for the unchanging PACAP serum levels following aerobic training in migraine patients is neuroprotective and neurotrophic role of PACAP. PACAP is expressed throughout the central nervous system (CNS), such as the hypothalamus, hippocampus, cerebellum, and sensory neurons (Arimura et al., 1994; Hannibal, 2002; Sundler et al., 1996), and this indicates its polyotropic functions in the CNS. PACAP has been shown to act as a nerve hormone, neurotransmitter, and neurotrophic agent (Gonzalez, Basille, Vaudry, Fournier, & Vaudry, 1997; Lioudyno, Skoglösa, Takei, & Lindholm, 1998; Vaudry et al., 2000). In the adult brain, PACAP prevents apoptotic cell death and can be a regenerative factor in various pathological conditions (Morio, Tatsuno, Hirai, Tamura, & Saito, 1996). Therefore, considering other roles and functions of PACAPs in the central nervous system, it seems that in migraines, the levels of this protein, on the one hand, are affected by migraine disease, and on the other hand, it requires different expressions for its other functions. Taking this into account, exercise training can affect PACAP roles in the nervous system. In general, it seems that the impact of aerobic training on PACAP in migraine patients will be different from that in healthy people. Therefore, in order to better determine the role of physical activity, such as aerobic training on PACAP in migraines, it is better to study the PACAP level in target tissues and local sites.

For the first time, we studied the effect of aerobic training with different intensities (MOD T and HIGH T) on serum SP levels in migraine patients. Interestingly, none of the aerobic training doses, medium, and high intensity, could have any effect on serum SP levels in migraine patients. The role of SP as a vasodilator in the brain has been mentioned in previous studies and was considered as a pain modulator (Beattie, Connor, & Hagan, 1995). In the clinical situations, it has also been shown that, when migraine attacks were triggered, the salivary SP level was increased in migraine patients as compared with the control group (Nicolodi & Del Bianco, 1990). The role of SP in migraine disease is well articulated in previous studies (Fusayasu, Kowa, Takeshima, Nakaso, & Nakashima, 2007; Jang, Park, Kho, Chung, & Chung, 2011). Nevertheless, the results of this study showed that aerobic training with moderate and high intensity did not have any significant effect on serum SP levels in migraine patients. In this regard, it expected that serum SP level would decrease after 8 weeks of aerobic training, but its serum levels did not change. The absence of change in SP after aerobic training may be attributed to other SP roles in the nervous system. Substance P has been shown to have neurotrophic effects and improve memory (Skoff, Zhao, & Adler, 2009). Electrophysiological studies have also shown that SP induces nigral dopaminergic neurons (Skoff et al., 2009). SP also increases dopamine release from dopamine terminals (Gayen, Goswami, & Mukhopadhyay, 2011), which may be involved in maintaining the integrity of the nerve population (Marolda et al., 2012). It also is involved in learning and memory, mood and anxiety, stress mechanisms, and pain (Xu, Xie, Li, Zhang, & Hou, 2013). Therefore, SP has many different functions in the nervous system, which may require different doses of it. However, the mechanisms underlying SP expression and its influence on migraine pain adaptation to exercise training are not entirely clear. Additional studies should be conducted to elucidate the role of SP in headache in migraine patients.

Conclusion

In summary, the present study showed that aerobic training with moderate and high intensities could significantly reduce migraine pain indices including headache intensity, duration, and frequency of attacks in migraine women. Although, role of PACAP and SP in beneficial effects of aerobic training on migraine indices remain unclear, however, these effects of aerobic training on migraine pain indices can be attributed to various mechanisms that require further studies. In addition, our results showed that the intensity of aerobic training was not the main factor in migraine pain modification, although both aerobic training protocols could be alternative non-pharmacological method to monitor the headache in women with migraine disorder.

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Author contributions

R. Eslami contributed in the data processing, statistical analysis, and drafted the manuscript; A. Parnow and Z. Pairo conceived of the study, participated in its design and coordination, run training protocols, and gathered data. P. Nikolaidis contributed in data analysis; and B. Knechtle contributed in final edition and managed. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors

Competing interests

The authors declare that there is no conflict of interest.

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Table 1: Demographic characteristics of subjects with different groups in initial state.

	CON (*n*=15) (mean±SD)	MOD T (*n*=15) (mean±SD)	HIGH T (*n*=15) (mean±SD)
Age (years)	32.44±5.74	38.41±6.20	25.16±6.08
Weight (kg)	61.98±10.55	69.75±14.42	57.12±10.84
BMI (kg/m^2)	24.10±4.42	26.93±5.65	21.00±3.1
VO_2max (ml/kg/min)	36.89±11.23	28.69±4.48	33.90±8.83
Body Fat (%)	28.54±6.89	29.87±10.19	21.60±6.57

CON: Control; MOD T; Moderate-intensity aerobic training; HIGH T: High-intensity aerobic training

Table 2: Aerobic training protocol with different intensities

Groups	Training Variables	Weeks							
		One	Two	tree	four	five	six	seven	eight
MOD T	Intensity (RPE)	8	12	12	13	14	14	15	15
	Intensity (%HRmax)	60	62	72	78	80	80	82	**82**
	Intensity (% VO_2max)	45	46	50	60	68	68	70	**70**
	Time (min)	7	18	25	30	35	40	40	40
	Work load (RPE*time)	56	216	300	390	490	560	600	600
HIGH T	Intensity	10	12	12	14	16	16	17	17
	Intensity (%HRmax)	65	79	82	88	92	92	92	**95**
	Intensity (% VO_2max)	55	70	72	80	83	88	88	**90**
	Time (min)	5	10	15	20	25	30	30	30
	Work load (RPE*time)	50	120	180	280	400	480	510	510

MOD T; Moderate-intensity aerobic training; HIGH T: High-intensity aerobic training

Table 3: The comparison of variables in pre and post time and between three groups (time effects and time*group interaction effects).

Variables	Time	CON (*n*=15) (mean±SD) Paired comparison (P-value)	MOD T (*n*=13) (mean±SD) Paired comparison (P-value)	HIGH T (*n*=12) (mean±SD) Paired comparison (P-value)	Time effects p-value	Interaction effect (time*group) P-value
Weight (kg)	**Pre:** **Post:**	61.98±10.55 63.03±11.42 P=0.160	69.75±14.42 67.40±13.29 *P<0.001	57.12±10.84 56.77±10.09 P=0.473	P=0.081, Eta-squared=0.098	$P<0.001 Eta-squared=0.404
BMI (kg/m²)	**Pre** **Post:**	24.10±4.42 24.50±4.74 P=0.171	26.93±5.65 26.01±5.14 *P<0.001	21.00±3.1 20.86±2.72 P=459	P=0.079, Eta-squared=1	$P<0.001 Eta-squared=0.401
VO₂max (ml/kg/min)	**Pre:** **Post:**	36.89±11.23 33.46±8.27 P=0.68	28.69±4.48 40.18±6.06 *P<0.001	33.90±8.83 48.48±11.06 *P≤0.001	#P<0.001, Eta-squared=0.539	$P<0.001 Eta-squared=0.538
Body Fat (%)	**Pre:** **Post:**	28.54±6.89 28.40±7.52 P=0.838	29.87±10.19 26.60±8.27 *P<0.01	21.60±6.57 20.85±5.95 *P<0.05	#P≤0.001, Eta-squared=0.312	$P<0.01 Eta-squared=0.306
WHR (score)	**Pre:** **Post:**	0.81±0.06 0.82±0.08 P=0.637	0.81±0.07 0.80±0.07 *P<0.01	0.78±0.07 0.78±0.07 P=0.111	#P=0.424, Eta-squared=0.021	P=0.214 Eta-squared=0.098
Pain intensity (VAS)	**Pre:** **Post:**	5.75±2.14 5.06±1.55 P=0.093	7.38±1.06 3.73±1.62 *P<0.001	5.79±1.58 4.22±2.07 *p≤0.001	#P<0.001, Eta-squared=0.684	$P<0.001 Eta-squared=0.458
Pain frequency (times per month)	**Pre:** **Post:**	8.66±3.00 7.44±2.35 P=0.056	7.54±3.11 2.27±1.34 *P<0.001	8.08±2.99 3.25±2.86 *P<0.001	#P<0.001, Eta-squared=0.746	$P≤0.01 Eta-squared=0.384
Pain Duration (hours per month)	**Pre:** **Post:**	10.51±4.83 8.62±3.96 *P<0.01	8.68±2.97 3.65±1.77 *P<0.001	7.29±3.41 4.04±2.32 *P<0.05	#P<0.001, Eta-squared=0.653	$P≤0.05 Eta-squared=0.205
PACAP (ng/ml)	**Pre:** **Post:**	72.44±0.98 72.49±0.96 P=0.833	71.88±0.29 72.00±0.44 P=0.469	72.00±0.44 72.32±0.98 P=0.177	P=0.234, Eta-squared:0.047	P=0.712 Eta-squared=0.022
SP (pg/ml)	**Pre:** **Post:**	937.85±2.90 937.87±2.86 P=0.449	938.51±1.73 938.96±1.48 P=0.219	938.57±1.90 938.55±1.90 P=0.629	P=0.241, Eta-squared:0.046	P=0.249 Eta-squared=0.089

CON: Control; MOD T; Moderate-intensity aerobic training; HIGH T: High-intensity aerobic training

* Significant pre-post different

\# Significant time effect

$ Significant time*group interaction effect

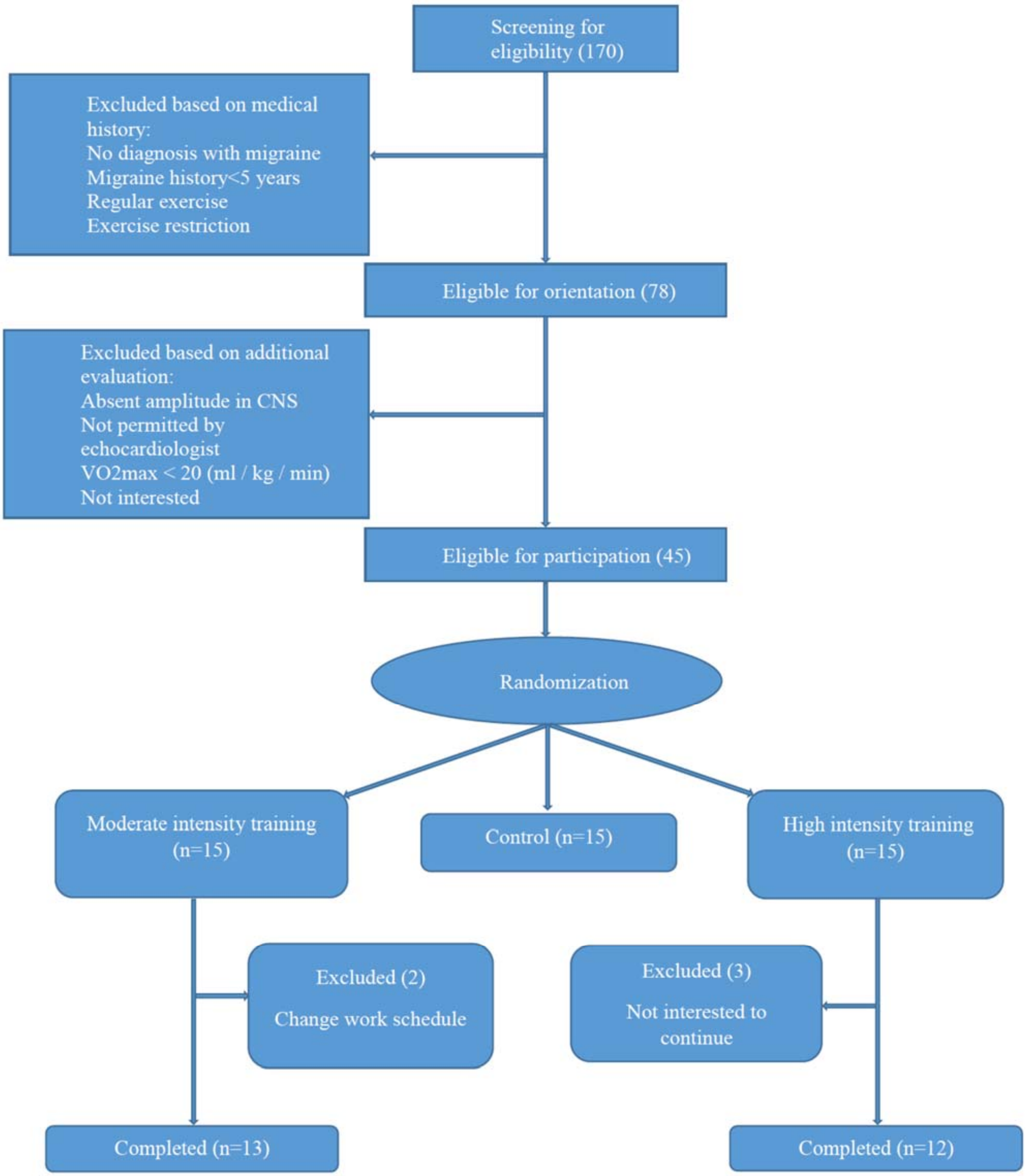

Figure 1: Participant recruitment flow chart.

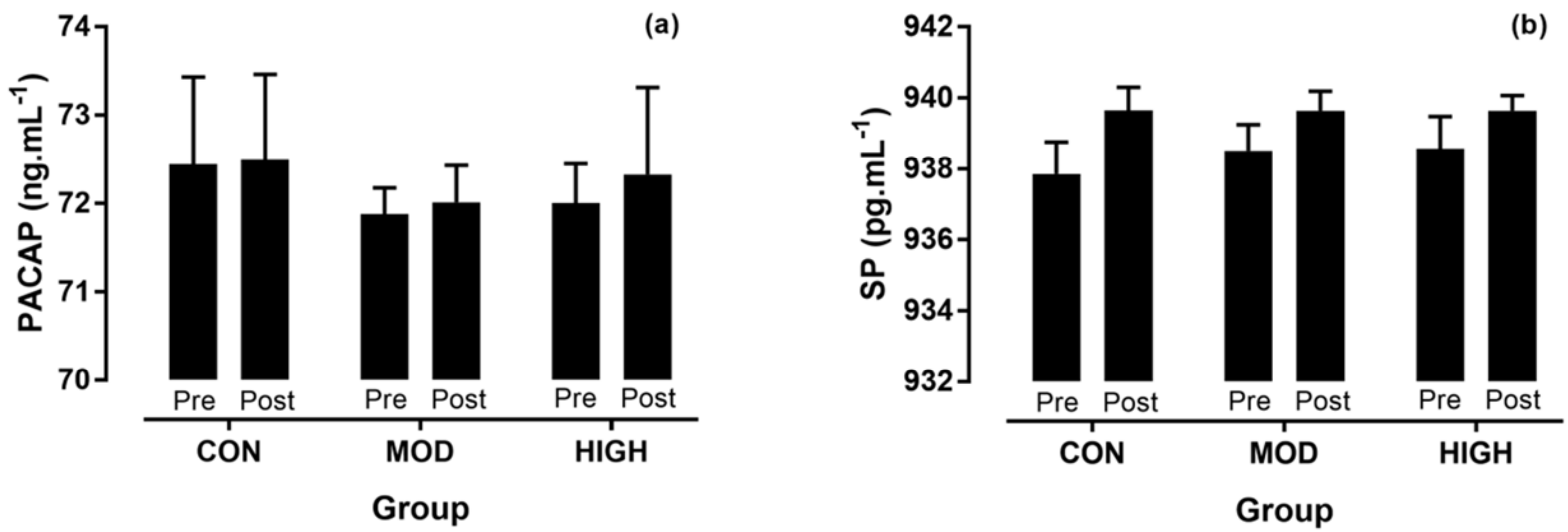

Figure2: PACAP (a) and SP (b) contents in three groups in pre and post training. CON: Control group; MOD: Moderate intense aerobic training group; HIGH: High intense aerobic training.